(5) Rejection of Claims 10-13 and 20-22 Under Section 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma;

(6) Rejection of Claims 1, 8, and 9 Under Section 103(a) as being allegedly unpatentable over Hammond, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, and Picataggio *et al.* (1992); and

(7) Rejection of Claims 25 and 26 Under Section 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), and Zimmer *et al.*

Each of the issues raised by the Examiner is discussed below. Applicants believe that the foregoing amendment and the following remarks respond completely to the objection and rejections. Applicants note that the Examiner has stated that claims 2, 7, 18, and 22 are allowed (see pages 2 and 10 of the Final Office Action and on the Office Action Summary page). Applicants agree with the Examiner and further believe that all pending claims are in condition for allowance.

Finally, Applicants submit herewith a Petition to withdraw finality of this Office Action. Applicants respectfully submit that the Examiner has improperly imposed a premature Final Office Action. Claim 1, which is now rejected under U.S.C. § 103(a) as being allegedly unpatentable over Hammond, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, and Picataggio *et al.* (1992), was previously amended to include limitations which should reasonably have been expected to be claimed, see MPEP 904 et seq. In addition, Claim 25, which was not previously rejected or amended, is now rejected under U.S.C. § 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), and Zimmer *et al.* Therefore, this second action on the merits of the Application presents new grounds of rejection to both claims 1 and 25 that were neither necessitated by Applicants' amendment of the claims nor based upon information submitted in an information disclosure statement filed during the period set forth in 37 C.F.R. 1.97(c). Accordingly, this Final Office Action is considered premature and improper [see MPEP 706.07(a)]. Applicants respectfully request reconsideration and withdrawal of the finality of this Office Action.

(1) Objection to Claim 1

Claim 1 has been objected to due to an improper dependency upon claim 3. In response, Applicants have amended pending claim 1 to recite an independent claim that incorporates all of the limitations of pending claim 3.

In view of the foregoing amendment, Applicants respectfully submit that the Examiner's objection is obviated and request that this objection be withdrawn.

(2) Rejection of Claim 16 Under Section 102(b) as being allegedly anticipated by Masuda *et al.* (1995)

Claim 16 stands rejected under 35 U.S.C. § 102(b), as allegedly anticipated by Masuda *et al.* Applicants respectfully traverse this rejection and submit that Masuda *et al.* do not teach each and every element of pending claim 16.

Masuda *et al.* (1995) Do Not Teach Applicants' Invention

Masuda *et al.* teach the cloning and characterization of the POX2 gene from *Candida maltosa* (*C. maltosa*). These authors also teach *C. maltosa* strains in which both alleles of either the POX2 or POX4 acyl-CoA oxidase isozyme genes have been disrupted. The POX2 disrupted strain (P2DD) was able to assimilate n-tetradecane, n-tetradecanol, and oleic acid however, the POX4 disrupted strain (P4DD) was defective for growth on these hydrophobic carbon sources (see Abstract and Table 1).

Independent claim 16 recites a transformed *Candida maltosa* characterized by disruption of no more than both POX4 genes encoding acyl-CoA oxidase whereby a β-oxidation pathway is functionally blocked. While Masuda *et al.* teach that a double disruption of the POX4 alleles in *C. maltosa* prevents growth on n-tetradecane, n-tetradecanol, and oleic acid, these authors do not teach that this POX4 mutant in unable to grow on other substrates that are also metabolized through the β-oxidation pathway. Masuda *et al.* do not assay for β-oxidation activity within the POX4 disrupted *C. maltosa*. Since Picataggio *et al.* [*Mol. Cell. Biol.* 11:4333-4339 (1991)] taught that different acyl-coA oxidase isozymes possess different substrate chain-length specificities, one of ordinary skill in the art might have expected that *Candida maltosa* contained additional POX genes for degradation of substrates other than those taught by Masuda *et al.* [see page 159, section (d)]. Indeed, Masuda *et al.* teach that the POX2 gene product may be important for assimilation of other hydrocarbons (see page 160, column 2). Consequently, the results of Masuda *et al.* fail to predict whether POX4 gene disruption alone is sufficient to result in a functional block of the β-oxidation pathway such that alkane and fatty acid substrates are converted to their corresponding dicarboxylic acids. Applicants were the first to make the observation that disruption of no more than both POX4 alleles was sufficient to render *C. maltosa* functionally blocked in the β-oxidation pathway.

Applicants submit respectfully that Masuda *et al.* do not teach each and every element of the invention as claimed. In view of the foregoing remarks, Applicants respectfully submit that claim 16 is not anticipated by Masuda *et al.* and request that this rejection be reconsidered and withdrawn.

(3) Rejection of Claims 8-23 and 27 Under Section 103(a) as being allegedly unpatentable over Picataggio *et al.* (5,254,466), in view of Picataggio *et al.* (1992), Masuda *et al.* (1995), Zimmer *et al.*, and Schunck *et al.* (1989)

Pending claims 8-23 and 27 stand rejected under Section 103(a) as being allegedly unpatentable over Picataggio *et al.* (5,254,466), in view of Picataggio *et al.* (1992), Masuda *et al.* (1995), Zimmer *et al.*, and Schunck *et al.* (1989). Applicants respectfully remind the Examiner that claims 18 and 22 have been allowed (see pages 2 and 10 of the Final Office Action). Therefore, Applicants presume that claims 18 and 22 have been mistakenly included in this rejection and are not discussed further. Applicants respectfully traverse this rejection and submit that this combination of

references in no way teaches or suggests Applicants' invention and, therefore, fails to establish a *prima facie* case of obviousness. Accordingly, Applicants request respectfully that the rejection be reconsidered and withdrawn.

(a) Discussion of the Cited References

(i) Picataggio *et al.* (5,254,466): Picataggio *et al.* ('466) teach processes to site-specifically modify the genome of *Candida tropicalis* (*C. tropicalis*). These authors also teach processes for completely blocking the β-oxidation pathway in *C. tropicalis* at its first reaction step by disrupting the chromosomal POX4A, POX4B and POX5 genes (column 6, lines 38-50 and Examples 8 and 14). In addition, Picataggio *et al.* ('466) teach processes for producing substantially pure omega-dicarboxylic acids in substantially quantitative yield by culturing a *C. tropicalis* strain in which the POX4A gene, POX4B gene and both POX5 genes have been disrupted (column 6, lines 53-60 and Example 17). Finally, this reference teaches that disruption of both POX4 genes alone results in only a 50% reduction of the β-oxidation pathway in *C. tropicalis* (see Figure 4, strain H43 and Example 11) and that both copies of both POX4 and POX5 genes are required to completely block this pathway (see Figure 4, strain H5343).

(ii) Picataggio *et al.* (1992): Picataggio *et al.* (1992) teach amplification of the cytochrome P450 monooxygenase (P450alk1) and the NADPH-oxidoreductase (CPR) genes in a *C. tropicalis* strain that comprises disruption of the POX4 and POX5 genes so that the β-oxidation pathway is blocked and alkane and fatty acid substrates are re-directed to the ω-oxidation pathway (see Abstract, page 894, column 2, and page 895, column 1). These authors teach that this *C. tropicalis* strain has an increased ω-hydroxylase activity and a substantial increase in productivity, resulting in an efficient bioconversion for the rapid production of dicarboxylic acids in high yield (see page 894, column 2 and pages 895-896).

(iii) Masuda *et al.* (1995): Masuda *et al.* teach transformed *C. maltosa* strains that have both POX2 genes or both POX4 genes disrupted. As discussed in section 2 above, Masuda *et al.* teach a double POX4 allele disruption in *C. maltosa* prevents its growth on n-tetradecane, n-tetradecanol, and oleic acid. These authors also teach that while the POX2 gene product was not essential for growth on n-tetradecane, n-tetradecanol, and oleic acid, it may regulate the function of the POX4 gene product or may be involved in assimilation of other carbon sources (see page 160, column 2). Masuda *et al.* do not teach that the β-oxidation pathway is functionally blocked in the POX4 disrupted strain. Consequently, the results of Masuda *et al.* fail to predict whether POX4 gene disruption alone is sufficient to result in a functional block of the β-oxidation pathway such that alkane and fatty acid substrates are converted to their corresponding dicarboxylic acids. As discussed in section 2 above, Masuda *et al.* (1995) simply fail to teach or suggest the necessary requirements to functionally block the β-oxidation pathway in *C. maltosa*.

(iv) Zimmer *et al.*: Zimmer et *al.* teach co-expression in *Saccharomyces cerevisiae* of *C. maltosa* NADPH-cytochrome P450 reductase with one of each of the following *C. maltosa* cytochrome P450 forms: P450Cm1, P450Cm2, and P450Alk2A using a multicopy plasmid containing two independent expression cassettes encoding these respective genes under the control of a galactose-inducible *GAL10* promoter (see Figure 1).

(v) Schunck *et al.* (1989): Schunck *et al.* teach the preparation of an n-alkane grown *C. maltosa* cDNA library and the cloning and sequence analysis of the alkane hydroxylating cytochrome P-450 ($P450_{alk}$) cDNA (see entire document). Schunck *et al.* teach that the deduced amino acid sequence of the *C. maltosa* $P450_{alk}$ protein is approximately 60% similar to that of *C. tropicalis* (see page 847 and Table 1). However, these authors teach that further examination is required to determine whether the $P450_{alk}$ of *C. maltosa* is the orthologous gene to that of *C. tropicalis* since the results of Loper *et al.* (1985) and Sokolov *et al.* (1986) indicate that more than just the P-450 form is induced in both *C. maltosa* and *C. tropicalis* when grown on n-alkanes (see page 848).

(b) Picataggio *et al.* (5,254,466) Do Not Render Obvious the Invention of Claims 8-23 and 27

As discussed above, Picataggio *et al.* ('466) teach that disruption of both copies of both POX4 and POX5 genes are required to completely block the β-oxidation pathway in *C. tropicalis* (see Figure 4, strain H5343 and Example 11). This reference is silent on the requirements of a *C. maltosa* strain to efficiently produce mono- and dicarboxylic acids. Picataggio *et al.* ('466) certainly do not teach or suggest a transformed *C. maltosa* comprising 1) an enhanced alkane hydroxylating activity, 2) a functionally blocked β-oxidation pathway arising from disruption of both POX4 genes, or 3) an enhanced alkane hydroxylating activity and a functionally blocked β-oxidation pathway as recited in Applicants' claims 10-13, 16, 17, 20 and 21. These authors also fail to teach or suggest methods for the enhanced bioproduction of C_6 to C_{22} mono- and di-carboxylic acids comprising such a transformed *C. maltosa* as recited in Applicants' claims 8, 9, 14, 15, 19, and 23. Finally, these authors do not teach or suggest either 1) a pSW84 plasmid comprising expression cassettes for both cytochrome p450 Alk1-A and cytochrome P450-NADPH reductase plus an ade1 selectable marker, or 2) a pSW87 plasmid comprising expression cassettes for both cytochrome P450 Alk3-A and cytochrome P450-NADPH reductase plus a his5 selectable marker as recited in Applicants' claim 27. Absent such a disclosure, Picataggio *et al.* ('466) cannot possibly render obvious Applicants' claimed invention.

(c) Picataggio *et al.* (1992), Masuda *et al.* (1995), Zimmer *et al.*, and Schunck *et al.* (1989) Do Not Correct the Deficiencies of Picataggio *et al.* (5,254,466)

On pages 6-8 of the first Office Action (Paper No. 9), mailed August 4, 1999, the Examiner alleged that the combination of cited references would have rendered obvious the claimed invention to enhance production of long chain mono- and dicarboxylic acids in *C. maltosa* to one of ordinary skill in the art simply by following the guidance of Picataggio *et al.* (1992) with the expectation of achieving the same results in *C. maltosa* as Picataggio *et al.* (1992) did with *C. tropicalis*. On page 7, the Examiner contends that Zimmer *et al.* would have provided the motivation to select *C. maltosa* as a host species based upon their disclosure that the P450 forms "probably developed in adaptation of the microorganism to the utilization of different hydrocarbons" and that Schunck *et al.* would have provided the expectation of success because they teach the sequence of the alkane hydroxylating cytochrome P450 of *C. maltosa* and discuss its level of similarity to *C. tropicalis*.

Applicants respectfully disagree and submit that the disclosure of Picataggio *et al.* (1992) would not serve as a guide for one of ordinary skill in the art at the time the invention was made to modify a *C. maltosa* cell since their teachings relate only to *C. tropicalis* and require that both POX4 and POX5 genes be disrupted. Disruption of both POX4 genes alone in *C. tropicalis* was insufficient to enable efficient production of dicarboxylic acids. Furthermore, Picataggio *et al.* (1992) offer no guidance regarding the requirements to achieve efficient production of dicarboxylic acids in *C. maltosa*.

While Masuda *et al.* teach that genetic disruption of both POX4 genes prevents the growth of *C. maltosa* on tetradecane, n-tetradecanol, and oleic acid, these authors do not teach that this POX4 mutant in unable to grow on other substrates that are also metabolized through the β-oxidation pathway. Masuda *et al.* also fail to teach or suggest whether the POX4 mutant would convert tetradecane, n-tetradecanol, oleic acid or any other substrates to the corresponding dicarboxylic acids. Since Picataggio *et al.* [*Mol. Cell. Biol.* 11:4333-4339 (1991)] taught that different acyl-coA oxidases isozymes possess different substrate chain-length specificities and that both POX4 and POX5 gene disruptions were required in *C. tropicalis*, one of ordinary skill in the art at the time the invention was made might have expected that *C. maltosa* contained additional POX genes for degradation of substrates other than those taught by Masuda *et al.* Indeed, Masuda *et al.* teach that the POX2 gene product may be important for assimilation of other hydrocarbons. Consequently, the results of Masuda *et al.* fail to predict whether POX4 gene disruption alone is sufficient to result in a functional block of the β-oxidation pathway such that alkane and fatty acid substrates are converted to their corresponding dicarboxylic acids.

While Zimmer *et al.* state that the P450 multigene family probably developed in *C. maltosa* in adaptation to use different hydrocarbons, these authors do not teach the roles of these P450 family members or their importance in the growth properties of *C. maltosa* on different long chain n-alkanes and fatty acids. Furthermore, since Zimmer *et al.* suggest that different substrate specificities of individual P450 forms determine the growth properties of *C. maltosa* on different long chain n-alkanes and fatty acids (see page 620, first paragraph), one of ordinary skill in the art would not know which P450 form(s) to use in *C. maltosa* based upon the teaching of Zimmer *et al.*, alone or in combination with the other cited references.

With regard to Schunck *et al.*, these authors merely teach a 60% similarity between the *C. maltosa* $P450_{alk}$ deduced amino acid sequence and that previously characterized in *C. tropicalis*. As Applicants discussed in their previous reply, *C. maltosa* is recognized as a separate species distinct from *C. tropicalis* on the basis of physiological, morphological and immunological properties and was clearly distinguished from *C. tropicalis* by insignificant DNA reassociation kinetics (see page 10 of Applicants' reply submitted January 4, 2000). Therefore, one of ordinary skill in the art would not conclude that a 60% similarity of $P450_{alk}$ protein sequences between C. *tropicalis* and *C. maltosa* equates to similarity between the organisms as a whole. Given that *C. maltosa* and *C. tropicalis* are recognized as distinct and separate microorganisms in the art and in light of the uncertainty of the POX gene requirement in *C. maltosa* for assimilation of various hydrocarbon sources set forth by Masuda *et al.*, it would not have been obvious to one of ordinary skill in the art which genetic

alteration(s) would be required to enhance alkane hydroxylating activity and/or block the β-oxidation pathway in *C. maltosa* based upon the observations of Picataggio *et al.* with *C. tropicalis*.

The Examiner also alleged that Applicants' plasmids as recited in claim 27 would have been rendered "obvious in view of the teachings of coexpression of P450 monooxygenase and P450 reductase" in *S. cerevisiae* by Zimmer *et al.* (see pages 7-8, bridging sentence, first Office Action, Paper No. 9). While Zimmer *et al.* teach co-expression of the *C. maltosa* NADPH-cytochrome P450 reductase with *C. maltosa* cytochrome P450Cm1, P450Cm2, or P450Alk2A in *S. cerevisiae* using a multicopy plasmid containing two the independent expression cassettes under the control of the *GAL10* promoter, these authors do not teach or suggest either 1) a pSW84 plasmid comprising expression cassettes for both cytochrome p450 Alk1-A and cytochrome P450-NADPH reductase plus an ade1 selectable marker, or 2) a pSW87 plasmid comprising expression cassettes for both cytochrome P450 Alk3-A and cytochrome P450-NADPH reductase plus a his5 selectable marker as recited in Applicants' claim 27. None of the other cited references teach or suggest how to make the plasmids as claimed. Therefore, this cited combination of references fails to render obvious Applicants' claimed invention.

In summary, none of the cited references, either alone or in combination, teach or suggest Applicants' claimed invention. Applicants were the first to demonstrate that POX4 gene disruption alone was sufficient to functionally block the β-oxidation pathway in *C. maltosa*. Therefore, Applicants' results can only be regarded as surprising and unexpected. Furthermore, none of the references teach or suggest using a *C. maltosa* as claimed by Applicants in a method to enhance bioproduction of C_6 to C_{22} mono-and dicarboxylic acids or the plasmids as claimed. Only Applicants' Specification teaches these requirements and demonstrates their successful use.

Applicants respectfully submit that the cited combination of references relied upon by the Examiner fails to provide the motivation and expectation of success to one of ordinary skill in the art to modify the teachings of Picataggio *et al.* ('466) to obtain Applicants' claimed invention. Absent such a disclosure, the combination of Picataggio *et al.* ('466), with Picataggio *et al.* (1992), Masuda *et al.* (1995), Zimmer *et al.* and Schunck *et al.* (1989) cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 8-23 and 27. Accordingly, the rejection must be based on improper hindsight given the benefit of Applicants' disclosure. However, as discussed below, use of hindsight reconstruction of an invention using Applicant's teachings is improper.

(d) The Cited References Fail to Enable Applicants' Claimed Invention

As discussed above, none of the prior art cited by the Examiner teach or suggest Applicants' claimed invention. At best, the Examiner's position poses an "obvious to try" situation. However, the Federal Circuit Court has, on numerous occasions, noted that while something may be obvious to try, it may not be obvious under 35 U.S.C. § 103. The proper standard is whether the prior art would have suggested to one of ordinary skill in the art that the invention should be carried out and would have a reasonable likelihood of success, viewed in light of the prior art. *In re Dow Chemical Company*, 5 USPQ 2d 1529, 1531 (Federal Circuit, 1988). "Both the suggestion and the expectation of success must be founded in the prior art, not in Applicants' disclosure." Id. In this case the cited

combination of references simply does not suggest to one of ordinary skill in the art that Applicants' claimed invention could be achieved with a reasonable likelihood of success.

Indeed, Picataggio *et al.* ('466), taken with Picataggio *et al.* (1992) and Masuda et al. (1995), actually teach away from the claimed invention. As stated above, both Picataggio *et al.* references teach that both POX4 and POX5 genes must be disrupted to functionally block the β-oxidation pathway in *C. tropicalis*. Masuda *et al.* (1995) teach that POX2 may be important for regulating POX4 gene product and/or assimilation of other hydrocarbon sources. Zimmer *et al.* and Schunck *et al.* offer no instructions or guidance to rectify this teaching away. Based upon these teachings, the ordinary artisan would not expect that disruption of no more than both POX4 genes would be successful to functionally block the β-oxidation pathway and enhance the alkane hydroxylating activity and bioproduction of C_6 to C_{22} mono- and dicarboxylic acids in *C. maltosa.*

Statements in a prior art reference that teach away from the claimed invention are an indicia of non-obviousness. Such disclosures that diverge from and teach away from the invention cannot be disregarded. E.g. Gillette Co. V. S. C. Johnson & Sons, Inc., 16 USPQ2d 1923, 1927 (Fed. Cir. 1990)

(e) The Rejection is Based on Improper Hindsight

As discussed above, none of the references relied upon teach or suggest the claimed invention. Accordingly, the rejection must be based on improper hindsight given the benefit of Applicants' disclosure. However, use of hindsight reconstruction of an invention using Applicant's teachings is clearly improper.

In this case, nothing in the art cited by the Examiner teaches or suggests Applicants' claimed invention. Picataggio *et al.* ('466), taken with Picataggio *et al.* (1992) and Masuda et al. (1995), actually teach away from the claimed invention.

(f) Applicants Have Demonstrated an Unexpected Result

Applicants have demonstrated that POX4 gene disruption alone is sufficient to functionally block the β-oxidation pathway in *C. maltosa* to enhance its alkane hydroxylating activity and bioproduction of C_6 to C_{22} mono- and dicarboxylic acids. Nothing in the art cited by the Examiner teaches or suggests the extent of success demonstrated by Applicants. Therefore, Applicants have established a surprising and unexpected result. Accordingly, Applicants submit that the cited combination of references fails to establish a *prima facie* case of obviousness. Therefore, Applicants request respectfully that the rejection be reconsidered and withdrawn.

(4) Rejection of Claims 3-6 Under Section 103(a) as being allegedly unpatentable over Cregg *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma

Pending claims 3-6 stand rejected under Section 103(a) as being allegedly unpatentable over Cregg *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma. Applicants respectfully traverse this rejection and submit that the combination of references in no way teaches or suggests Applicants' invention and, therefore, fails to establish a *prima facie* case of obviousness. Accordingly, Applicants request respectfully that the rejection be reconsidered and withdrawn.

(a) Discussion of the Cited References

(i) Cregg *et al.*

Cregg *et al.* provide a review of Aox1 promoter controlled heterologous gene expression in *Pichia pastoris* (*P. pastoris*). These authors describe the use of such an expression system to produce and purify high levels of proteins of interest (see Abstract). Cregg *et al.* teach that the goal of most expression efforts in *P. pastoris* is the secretion of foreign protein of commercial interest (see page 906, column 2, second paragraph and page 909 Conclusion section). In addition, Cregg *et al.* teach that the *P. pastoris* expression system is unpredictable from protein to protein (see Table 1). Cregg *et al.* fail to teach or suggest co-expression of two genes or the use of the *P. pastoris* Aox1 expression system to enhance the alkane hydroxylating activity of *P. pastoris* upon contact with at least one C_6 to C_{22} straight chain hydrocarbon as required by Applicants' claims.

(ii) Takagi *et al.*, Takagi (Ohkuma *et al.*) and, Ohkuma:

Takagi *et al.* (D00481) teach the *C. maltosa* gene for cytochrome P-450alk.

Takagi (Ohkuma *et al.*; X55881) teach the *C. maltosa* ALK2-A and ALK3-A genes for n-alkane inducible cytochrome P-450.

Ohkuma (D12716) teaches the *C. maltosa* ALK4 gene for n-alkane inducible cytochrome P-450.

Ohkuma (D12717) teaches the *C. maltosa* ALK5-A gene for n-alkane inducible cytochrome P-450.

Ohkuma (D12718) teaches the *C. maltosa* ALK6-A gene for n-alkane inducible cytochrome P-450.

Ohkuma (D12719) teaches the *C. maltosa* ALK7 and ALK8 genes for n-alkane inducible cytochrome P-450.

Ohkuma (D25327) teaches the *C. maltosa* NADPH cytochrome P-450 reductase gene.

(b) Cregg *et al.* Do Not Render Obvious the Invention of Claims 3-6

Applicants' claims 3-6 relate to a transformed *P. pastoris* comprising a nucleic acid encoding a cytochrome P450 monooxygenase and, optionally, a nucleic acid encoding a cytochrome P450 reductase, wherein each nucleic acid is operably linked to a suitable regulatory element (a *P. pastoris* Aox1 promoter in claims 3-5), such that alkane hydroxylating activity is enhanced upon contact with at least one C_6 to C_{22} straight chain hydrocarbon.

The Examiner alleges on page 4 of the Final Office Action that it would have been obvious to make a transformed *P. pastoris* strain according to claims 3-6 since the rationale, reagents, and process steps for making this strain were known. The Examiner contends that Cregg *et al.* provide the motivation to make such a transformed strain since they teach the advantages of expressing foreign genes in *P. pastoris*. Applicants respectfully disagree. The teachings of Cregg *et al.* are concerned with the use of *P. pastoris* Aox1 gene expression systems for large scale protein production. Cregg *et al.* fail to teach or suggest co-expression of two genes or the use of the *P. pastoris* Aox1 expression system to enhance the alkane hydroxylating activity of *P. pastoris* upon

contact with at least one C_6 to C_{22} straight chain hydrocarbon as required by Applicants' claims. Cregg *et al.* do not suggest, much less teach the reconstitution of a *Candida maltosa* alkane hydroxylating activity in *P. pastoris*. These authors certainly do not provide any instruction on how to make and use such a strain or that such a strain would possess an enhanced alkane hydroxylating activity.

As previously discussed in Applicants' reply submitted January 4, 2000 (see page 8, last paragraph), expression systems are protein-specific. Specifically, it is not possible to predict functional expression of a given gene in a given system. Cregg *et al.* is a good example of this high uncertainty of heterologous protein expression specifically for *P. pastoris*. Table 1 of Cregg *et al.* (page 905) shows that protein expression levels in their *P. pastoris* expression system range from 0.3 g/L to 12 g/L protein. These data clearly show high variability from protein to protein, indicating the unpredictability of producing foreign proteins in this system. For example, with hepatitis B surface antigen, 0.3 g/L corresponds to 3% of protein, whereas with bovine lysozyme, 0.3 g/L corresponds to 60% of protein; with human epidermal growth factor, 80% of protein corresponds to 0.5 g/L, whereas with invertase, 80% of protein corresponds to 2.5 g/L (see Table 1). Since the *P. pastoris* expression system is unpredictable for expressing proteins, one of ordinary skill in the art would not look to Cregg *et al.* for instructions to express a protein or combination of proteins and reconstitute a *C. maltosa* alkane hydroxylating activity in *P. pastoris*. The ordinary artisan would simply have no expectation that such a system would work.

(c) Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma Do Not Correct the Deficiencies of Cregg *et al.*

Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma simply disclose nucleic acid and amino acid sequences. These references do not disclose how to enhance alkane hydroxylating activity in the *P. pastoris* expression system of Cregg *et al.* nor do these references provide the motivation to combine their sequence disclosures with the teachings of Cregg *et al.* to obtain Applicants' claimed invention. Absent such a teaching, these references cannot possibly correct the deficiencies of Cregg *et al.* outlined above. Nothing in the art cited by the Examiner teaches or suggests the invention as claimed. Applicants were the first to make this unexpected discovery. Accordingly, the rejection must be based on improper hindsight given the benefit of Applicants' disclosure. However, as discussed in section 3 above, use of hindsight reconstruction of an invention using Applicant's teachings is clearly improper. Therefore, the cited combination of references cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 3-6. Applicants request respectfully that the rejection be reconsidered and withdrawn.

(5) Rejection of Claims 10-13 and 20-22 Under Section 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma

Pending claims 10-13 and 20-22 stand rejected under Section 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma. Applicants respectfully remind the Examiner that claim 22 is allowed (see pages 2 and 10 of the

Final Office Action), therefore, independent claim 22 is considered to have been mistakenly included within this rejection and is not discussed further.

Applicants respectfully traverse this rejection and submit that the cited combination of references in no way teaches or suggests Applicants' claimed invention and, therefore, fails to establish a *prima facie* case of obviousness. Accordingly, Applicants request respectfully that the rejection be reconsidered and withdrawn.

(a) Discussion of the Cited References

(i) Kasuske *et al.*: This reference teaches homologous vector plasmids and electroporation transformation conditions for *C. maltosa* using both integrative and autonomously replicating plasmids (see entire document). Kasuske *et al.* do not teach or suggest a transformed *C. maltosa* characterized by an enhanced alkane hydroxylating activity and/or a β-oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase, as required by Applicants' claims.

(ii) Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma: As discussed in section 4 above, Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma teach the nucleic acid coding sequences and deduced amino acid sequences for the *C. maltosa* cytochrome P-450alk, ALK2-A, ALK3-A, ALK4, ALK5-A, ALK6-A, ALK7, and ALK8 genes and the *C. maltosa* NADPH cytochrome P-450 reductase gene. These authors do not provide any instructions or incentive to combine the sequences of their disclosures with Kasuske *et al.* to obtain Applicants' invention as claimed.

(b) Kasuske *et al.* Do Not Render Obvious the Invention of Claims 10-13 and 20-22

Applicants' claims relate to a transformed *C. maltosa* characterized by an enhanced alkane hydroxylating activity and/or a β-oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase. The reference cited by the Examiner does not teach or suggest the invention defined by claims 10-13 and 20-22. The reference is deficient because it discloses only plasmid vectors and conditions for transformation of *C. maltosa*, and fails to enable the transformed *C. maltosa* characterized by an enhanced alkane hydroxylating activity and/or functionally blocked β-oxidation pathway observed by Applicants. Absent such a disclosure, Kasuske *et al.* cannot possibly render *prima facie* obvious the invention defined by Applicants' claims.

(c) Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma Do Not Correct the Deficiencies of Kasuske *et al.*

Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma are limited to nucleic acid and deduced amino acid sequences disclosures. These references do not provide any other instructions. In particular, these references are completely silent on how to modify the vectors of Kasuske *et al.* to obtain Applicants' claimed invention. Accordingly, these references, either alone or in combination, cannot possibly correct the deficiencies of Kasuske *et al.*

Nothing in the art cited by the Examiner teaches or suggests a transformed *C. maltosa* characterized by an enhanced alkane hydroxylating activity and/or a β-oxidation pathway

functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase. Applicants were the first to make this unexpected discovery. Accordingly, the rejection must be based on improper hindsight given the benefit of Applicants' disclosure. However, as discussed in section 3 above, use of hindsight reconstruction of an invention using Applicant's teachings is clearly improper. Therefore, the cited combination of references cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 10-13 and 20-22. Applicants request respectfully that the rejection be reconsidered and withdrawn.

(6) Rejection of Claims 1, 8, and 9 Under Section 103(a) as being allegedly unpatentable over Hammond, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, and Picataggio *et al.* (1992)

Claims 1, 8, and 9 stand rejected under Section 103(a) as being allegedly unpatentable over Hammond, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, and Picataggio *et al.* (1992). On page 6 of the Final Office Action, the Examiner's discussion of the rejection of method claims 8 and 9 is directed to claims 9 and 10. Since Applicants' claim 10 is a composition of matter claim, Applicants presume that the Examiner's arguments are mistakenly directed to claims 9 and 10, instead of claims 8 and 9. Applicants herein address their reply to the Examiner's rejection of method claims of 8 and 9. Applicants have amended claim 1 and claims 1, 8, and 9 are pending in the instant amendment.

Applicants respectfully traverse this rejection and submit that the cited combination of references in no way teaches or suggests Applicants' invention and, therefore, fails to establish a *prima facie* case of obviousness. Accordingly, Applicants request respectfully that the rejection be reconsidered and withdrawn.

(a) Discussion of the Cited References

(i) Hammond: This reference teaches processes for "preparing a dicarboxylic acid from a hydrocarbon-chain containing substrate, in which a solid support or a non-aqueous gel support having active cells of a microorganism producing dicarboxylic acid immobilized within or on it is contacted with a homogenous non-aqueous liquid containing the substrate by moving said liquid through a packed bed comprising said support, said liquid containing water and an oxygen source" (see pages 3-4, bridging paragraph). Hammond teaches that the microorganism may be a hydrocarbon using yeast selected from the genera *Candida*, *Debaryomyces*, *Saccharomycopsis*, *Pichia* and *Torulopsis* (see pages 8-9, bridging paragraph), however, Hammond's teachings are centered solely upon a particular mutant *C. maltosa* strain which is neither described genotypically nor publicly available (see page 10, lines 14-20). Therefore, Hammond does not teach how to make this mutant *C. maltosa* strain or even what mutation(s) is required in order for this strain to produce dicarboxylic acids. Hammond is completely silent on the genetic requirements of this mutant strain, let alone what modifications are or would be necessary to enhance its alkane hydroxylating activity.

(ii) Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma: As discussed in sections 4 and 5 above, Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma teach the nucleic acid coding sequences and deduced amino acid sequences for the *C. maltosa* cytochrome P-450alk, ALK2-A, ALK3-A,

ALK4, ALK5-A, ALK6-A, ALK7, and ALK8 genes and the *C. maltosa* NADPH cytochrome P-450 reductase gene. These references do not provide any instructions or incentive to combine the teachings of their disclosures with Hammond to obtain Applicants' invention as claimed.

(iii) Picataggio *et al.* (1992): As discussed in section 3 above, Picataggio *et al.* (1992) teach amplification of the cytochrome P450alk1 and CPR genes in a *C. tropicalis* strain that comprises disruption of the POX4 and POX5 genes such that the β-oxidation pathway within this *C. tropicalis* is blocked and alkane and fatty acid substrates are re-directed to the ω-oxidation pathway (see Abstract, page 894, column 2, and page 895, column 1). These authors teach that this *C. tropicalis* strain has an increased ω-hydroxylase activity and a substantial increase in productivity, resulting in an efficient bioconversion for the rapid production of dicarboxylic acids in high yield (see page 894, column 2 and pages 895-896). Picataggio *et al.* (1992) fail to teach or suggest how to modify the teaching of Hammond with the teaching of their disclosure, either alone or in combination with the teachings of Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma, to obtain Applicants' invention as claimed.

(b) Hammond Does Not Render Obvious the Invention of Claims 1, 8, and 9

Applicants' claims relate to methods for the bioproduction of a C_6 to C_{22} mono- or di-carboxylic acid comprising a) contacting, under aerobic conditions, a transformed *P. pastoris* or *C. maltosa* comprising a genetically-engineered, enhanced alkane hydroxylating activity with at least one C_6 to C_{22} straight chain hydrocarbon; and b) recovering the C_6 to C_{22} mono- and di-carboxylic acids.

The Examiner alleges on pages 5 and 6 of the Final Office Action that it would have been obvious to produce or enhance the production of C_6 to C_{22} mono- and dicarboxylic acids according to Applicants' claims 1, 8 and 9 since the rationale, reagents, and process steps were known. The Examiner relies upon the rejections under section 103(a) as applied to claims 3-6 (see section 4 above) and to claims 10-13, 20, and 21 (see section 5 above) to allege that Applicants' claimed transformed *P. pastoris* and *C. maltosa* strains are obvious. As Applicants have already discussed, these transformed yeasts are not obvious for the reasons outlined herein. As discussed above in sections 4 and 5 above, the Examiner has improperly reconstructed the yeasts of Applicants' claims 3-6, 10-13, and 20-22 using hindsight given the benefit of Applicants' disclosure. Since the yeasts of Applicants' claimed invention are novel and non-obvious, the claimed methods of using these yeasts are also novel and non-obvious.

The Examiner states on page 6 of the Final Office Action that one could use Applicants' genetically-engineered *C. maltosa* in the process described by Hammond to produce any mono- or dicarboxylic acid. Applicants respectfully disagree and submit that since Hammond fails to teach the genotype of the *Candida maltosa* he used in his disclosed methods and since this yeast is not publicly available, one of ordinary skill in the art would not have known the characteristics that permitted the yeast of Hammond to be used in his methods. Without this disclosure, Hammond fails to teach how to make the yeast of his invention and provides no instructions on the genotypic requirements of *C. maltosa* to produce dicarboxylic acids. Hammond certainly does not teach the

requirements of other yeasts, *i.e. Pichia pastoris*, in his method. Absent such a disclosure, Hammond cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 1, 8, and 9.

(c) Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, and Picataggio *et al.* (1992) Do Not Correct the Deficiencies of Hammond

Takagi *et al.*, Takagi (Ohkuma *et al.*) and Ohkuma only disclose nucleic acid and amino acid sequences. These references provide no other instructions or incentive to combine their teachings with those of Picataggio *et al.* (1992) and Hammond to obtain Applicants' invention as claimed. These references clearly fail to correct the deficiencies of Hammond.

Picataggio *et al.* teach that only disruption of both the POX4 and POX5 genes results in a functionally blocked β-oxidation pathway in *C. tropicalis* whereby alkane and fatty acid substrates are bioconverted for the rapid production of dicarboxylic acids in high yield. As discussed above in section 3, the results of Picataggio *et al.* would not serve as a guide for one of ordinary skill in the art to modify a *C. maltosa* cell to produce mono- or dicarboxylic acids since Masuda *et al.* (1995) teach that the POX2 gene of *C. maltosa* may play a role, either in regulating the *C. maltosa* POX4 gene product or in assimilating other carbon sources. Picataggio *et al.* do not correct the deficiencies of Hammond since these authors are only concerned with the genetic engineering of *C. tropicalis*, a different organism with different gene disruption requirements for dicarboxylic acid production. Picataggio *et al.* certainly do not teach what would be required to modify a *Pichia pastoris* cell, an organism even more dissimilar to *C. tropicalis* than *C. maltosa*.

Cregg *et al.*, as applied in combination with the cited gene bank references to the *Pichia pastoris* of Applicants' claims 3-6 in section 4 above, also fail to teach or suggest the *P. pastoris* genotype requirements for mono- and di-carboxylic acid production and enhancement. Applicants respectfully submit that one of ordinary skill in the art would not be motivated to prepare 1) a transformed *Pichia pastoris* according to claims 3-6 based upon the deficient teachings of Cregg *et al.* in view of Takagi *et al.*, Takagi (Ohkuma *et al.*) and Ohkuma (see section 4 above) or 2) a transformed *Candida maltosa* according to claims 10-13, 20, and 21 based upon the deficient teachings of Kasuske *et al.* in view of Takagi *et al.*, Takagi (Ohkuma *et al.*) and Ohkuma (see section 5 above) and expect reasonable success in using these transformed yeasts in a method to bioproduce C_6 to C_{22} mono- and di-carboxylic acids as recited in Applicants' claims 1, 8 and 9, without the guidance provided by Applicants' disclosure. Nothing in the art cited by the Examiner teaches Applicants' claimed invention. Only Applicants' Specification provides the incentive to make and use the transformed yeasts in the claimed methods to produce mono- and dicarboxylic acids.

Accordingly, the rejection must be based on improper hindsight given the benefit of Applicants' disclosure. However, as discussed in section 3 above, use of hindsight reconstruction of an invention using Applicant's teachings is clearly improper. Therefore, the cited combination of references cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 1, 8, and 9. Applicants request respectfully that the rejection be reconsidered and withdrawn.

(7) Rejection of Claims 25 and 26 Under Section 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), and Zimmer *et al.*

Pending claims 25 and 26 stand rejected under Section 103(a) as being allegedly unpatentable over Kasuske *et al.*, in view of Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), and Zimmer *et al.* Applicants respectfully traverse this rejection and submit that the cited combination of references in no way teaches or suggests Applicants' claimed invention and, therefore, fails to establish a *prima facie* case of obviousness. Accordingly, Applicants request respectfully that the rejection be reconsidered and withdrawn.

(a) Discussion of the Cited References

(i) Kasuske *et al.*: As discussed in section 5 above, Kasuske *et al.* teach homologous vector plasmids and electroporation transformation conditions for *C. maltosa* using both integrative and autonomously replicating plasmids (see entire document). These authors do not teach or suggest a DNA fragment comprising a) a first *C. maltosa* promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 monooxygenase and b) a second *C. maltosa* promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 reductase. Kasuske *et al.* do not teach or suggest the use of a *C. maltosa* PGK promoter in their plasmids.

(ii) Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma: As discussed in sections 4-6 above, Takagi *et al.*, Takagi (Ohkuma *et al.*), and Ohkuma disclose only the nucleic acid and deduced amino acid sequences for the *C. maltosa* cytochrome P-450alk, ALK2-A, ALK3-A, ALK4, ALK5-A, ALK6-A, ALK7, and ALK8 genes and the *C. maltosa* NADPH cytochrome P-450 reductase gene. These references provide no other instructions or suggestions.

(iii) Masuda *et al.* (1994): Masuda *et al.* teach the isolation and sequence of the PGK gene of *C. maltosa*, a gene that is significantly induced in cells grown on glucose but not in cells grown on n-tetradecane, n-tetradecanol, or oleic acid (see Figure 2). These authors teach the modification of this PGK gene's promoter by introducing a non-native restriction site by site directed mutagenesis (see Figure 3) to express an endogenous P450alk1 gene or a heterologous LAC4 gene when the cells are grown on glucose media (see Abstract, Figure 4, and page 416, columns 1 and 2, bridging paragraph). These authors do not teach or suggest a DNA fragment comprising a) a first *C. maltosa* promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 monooxygenase and b) a second *C. maltosa* promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 reductase.

(iv) Zimmer *et al.*: As discussed in section 3 above, Zimmer et *al.* teach co-expression of *C. maltosa* NADPH-cytochrome P450 reductase with each of the following *C. maltosa* cytochrome P450 forms: P450Cm1, P450Cm2, and P450Alk2A in *S. cerevisiae* using a multicopy plasmid containing the two independent expression cassettes under the control of a galactose-inducible *GAL10* promoter (see Figure 1). These authors fail to teach or suggest that a *C. maltosa* PGK promoter can be successfully substituted for the GAL10 promoter in their expression cassettes.

(b) Kasuske *et al.* Do Not Render Obvious the Invention of Claims 25 and 26

Kasuske *et al.* teach homologous vector plasmids and electroporation transformation conditions for *C. maltosa* using both integrative and autonomously replicating plasmids (see entire document). Kasuske *et al.* do not teach a DNA fragment comprising a) a first *C. maltosa* promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 monooxygenase and b) a second *C. maltosa* promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 reductase. These authors also fail to teach the use of a *C. maltosa* PGK promoter in their plasmids. Kasuske *et al.* certainly fail to teach or suggest a DNA fragment comprising a) a first *C. maltosa* PGK promoter which is operably linked to a gene encoding cytochrome P450 monooxygenase and b) a second *C. maltosa* PGK promoter operably linked to a gene encoding a *C. maltosa* cytochrome P450 reductase. Absent such a disclosure, Kasuske *et al.* cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 25 and 26.

(c) Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), and Zimmer *et al.* Do Not Correct the Deficiencies of Kasuske *et al.*

Takagi et *al.*, Takagi (Ohkuma *et al.*), and Ohkuma merely recite nucleic acid and amino acid sequences. Masuda *et al.* (1994) teach a promoter modification procedure that alters the nucleotide sequence at both the 3' end of PGK promoter and at the 5' end of the coding sequence to which it is ligated. In contrast, the present invention describes a precise fusion between the PGK promoter and the coding sequence to be expressed (Examples 2-4), with no additional, deleted, or altered nucleotides at the junction between the PGK promoter and *C. maltosa* gene to be expressed. Since it is well know in the art that even single base alterations in transcriptional control regions can have profound and unexpected functional effects, one of ordinary skill in the art would not have been motivated to modify the promoter of Masuda *et al.* (1994) to make the fusion constructs described by Applicants to express cytochrome P450 monooxygenase and reductase genes. Such precise fusion constructs are not taught or anticipated by Kasuske *et al.*, Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), or Zimmer *et al.*, either alone or together. Applicants were the first to make this discovery.

While Zimmer et *al.* teach a multicopy plasmid comprising a GAL10 promoter-NADPH-cytochrome P450 reductase expression cassette and a second expression cassette encoding one of cytochrome P450 forms: P450Cm1, P450Cm2, and P450Alk2A under the control of the GAL10 promoter for co-expression in *S. cerevisiae,* these authors fail to teach or suggest that a *C. maltosa* PGK promoter can be successfully substituted for the GAL10 promoter in their expression cassettes.

Applicants respectfully submit that Takagi *et al.*, Takagi (Ohkuma *et al.*), Ohkuma, Masuda *et al.* (1994), and Zimmer *et al.* do not teach or suggest Applicants' claimed invention. As discussed above, none of these references provide an incentive or the instructions to combine the teachings of their disclosure, either alone or in combination, to correct the deficient teaching of Kasuske *et al.* Nothing in the art cited by the Examiner teaches or suggests the invention as claimed. Applicants were the first to make this unexpected discovery. Accordingly, the rejection must be based on improper hindsight given the benefit of Applicants' disclosure. However, as discussed in section 3 above, use of hindsight reconstruction of an invention using Applicant's teachings is clearly

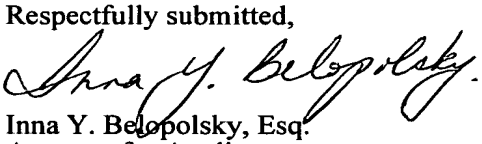
improper. Therefore, the cited combination of references cannot possibly render *prima facie* obvious the invention defined by Applicants' claims 25 and 26. Applicants request respectfully that the rejection be reconsidered and withdrawn.

Conclusion

In view of the foregoing amendments and remarks, Applicants submit that this application is in condition for allowance. Favorable reconsideration and an action passing this case to issue are therefore requested respectfully. If a telephone interview would be of assistance in advancing prosecution of this application, Applicants' attorney invites the Examiner to contact her at the number provided below.

E.I. DuPont de Nemours and Company
Legal-Patents
Barley Mill Plaza 11/1154
Wilmington, DE 19805
Telephone: (302) 992-4406
Facsimile : (302) 892-7949

Respectfully submitted,



Inna Y. Belopolsky, Esq.
Attorney for Applicants
Registration No. 43,319

Dated:

APPENDIX
U.S. Patent Application Serial No. 09/116,502
"Transformed Yeast Strains and Their Use for the Production of Monoterminal and Diterminal Aliphatic Carboxylates"
DuPont File No. CL-1035
Pending Claims

1. (Twice Amended) A method for the bioproduction of a C_6 to C_{22} mono- or di-carboxylic acid comprising

a) contacting, under aerobic conditions, a transformed *Pichia pastoris* characterized by a genetically-engineered alkane hydroxylating activity comprising

i) at least one copy of a foreign gene encoding cytochrome P450 monooxygenase; and, optionally,

ii) at least one copy of a foreign gene encoding cytochrome P450 reductase, each gene operably linked to a *Pichia pastoris* Aox1 promoter such that alkane hydroxylating activity is enhanced upon contact with at least one C_6 to C_{22} straight chain hydrocarbon,

with at least one C_6 to C_{22} straight chain hydrocarbon; and

b) recovering the C_6 to C_{22} mono- or di-carboxylic acid.

2. (Amended) The method of Claim 1, wherein the transformed *Pichia pastoris* is strain SW 64/65 identified as ATCC 74409; the at least one C_6 to C_{22} straight chain hydrocarbon is dodecane; and the recovered C_6 to C_{22} mono- or di-carboxylic acid is dodecanedioic acid.

3. (Amended One Time) A transformed *Pichia pastoris* comprising

a) at least one copy of a foreign gene encoding cytochrome P450 monooxygenase; and, optionally,

b) at least one copy of a foreign gene encoding cytochrome P450 reductase, each gene operably linked to *Pichia pastoris* Aox1 promoter such that alkane hydroxylating activity is enhanced upon contact with at least one C_6 to C_{22} straight chain hydrocarbon.

4. (Amended One Time) The transformed *Pichia pastoris* of Claim 3 wherein the foreign gene encoding cytochrome P450 monooxygenase is selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)).

5. (Amended One Time) The transformed *Pichia pastoris* of Claim 3 wherein the foreign gene encoding cytochrome P450 reductase is cytochrome P450 reductase (D25327 (SEQ ID NO:43)).

6. (Amended One Time) A transformed *Pichia pastoris* strain characterized by an enhanced alkane hydroxylating activity and comprising,

a) at least one DNA fragment from *Candida maltosa* ATCC 90677 selected from the group of DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37); and, optionally,

b) at least one DNA fragment from *Candida maltosa* ATCC 90677 encoding cytochrome P450 reductase,

each DNA fragment operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C_6 to C_{22} straight chain hydrocarbon.

7. A transformed *Pichia pastoris* strain SW64/65 identified as ATCC 74409.

8. (Amended One Time) A method for the enhanced bioproduction of C_6 to C_{22} mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, a transformed *Candida maltosa* characterized by a genetically-engineered, enhanced alkane hydroxylating activity with at least one C_6 to C_{22} straight chain hydrocarbon; and

b) recovering the C_6 to C_{22} mono- and di-carboxylic acids.

9. (Amended One Time) The method of Claim 8 wherein

a) the genetically-engineered, enhanced alkane hydroxylating activity arises from

i) at least one additional copy of the genes encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)); or

ii) at least one additional copy of the gene encoding cytochrome P450 reductase (D25327); or

iii) at least one additional copy of both the genes of i) and ii);

b) the at least one C_6 to C_{22} straight chain hydrocarbon is dodecane; and

c) the product recovered is dodecanedioic acid.

10. (Amended One Time) A transformed *Candida maltosa* comprising

a) at least one additional copy of an integrated gene encoding cytochrome P450 monooxygenase; or

b) at least one additional copy of an integrated gene encoding cytochrome P450 reductase; or

c) at least one additional copy of both the integrated gene encoding P450 monooxygenase and the integrated gene encoding cytochrome P450 reductase,

each integrated gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C_6 to C_{22} straight chain hydrocarbon.

11. (Amended One Time) The transformed *Candida maltosa* of Claim 10 wherein the genes encoding cytochrome P450 monooxygenase are selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)).

12. (Amended One Time) The transformed *Candida maltosa* of Claim 10 wherein the gene encoding cytochrome P450 reductase is cytochrome P450 reductase (D25327 (SEQ ID NO:43)).

13. (Amended One Time) A transformed *Candida maltosa* strain comprising

a) at least one DNA fragment from *Candida maltosa* (ATCC 90677) selected from the group of DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37), and

b) at least one DNA fragment from *Candida maltosa* (ATCC 90677) encoding cytochrome P450 reductase,

each gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C_6 to C_{22} straight chain hydrocarbon.

14. A method for the enhanced bioproduction of C_6 to C_{22} mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, transformed *Candida maltosa* characterized by a genetically-engineered, blocked β-oxidation pathway with at least one C_6 to C_{22} straight chain hydrocarbon; and

b) recovering the C_6 to C_{22} mono- and di-carboxylic acids.

15. The method of Claim 14 wherein the transformed *Candida maltosa* β-oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase.

16. (Amended One Time) A transformed *Candida maltosa* characterized by disruption of no more than both POX4 genes encoding acyl-CoA oxidase whereby a β-oxidation pathway is functionally blocked.

17. A transformed *Candida maltosa* characterized by a β-oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase using a single URA3 selectable marker.

18. A transformed *Candida maltosa* strain SW81/82 identified as ATCC 74431.

19. A method for the enhanced bioproduction of C_6 to C_{22} mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, transformed *Candida maltosa* characterized by

i) a genetically-engineered, enhanced alkane hydroxylating activity, and

ii) a genetically-engineered, blocked β-oxidation pathway, with at least one C_6 to C_{22} straight chain hydrocarbon; and

b) recovering the C_6 to C_{22} mono- and di-carboxylic acids.

20. (Amended One Time) A transformed *Candida maltosa* characterized by

a) an enhanced alkane hydroxylating activity arising from

i) at least one additional copy of a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)),

Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)), or

ii) at least one additional copy of a gene encoding cytochrome P450 reductase (D25327 (SEQ ID NO:43)), or

iii) at least one additional copy of both the genes i) and ii); and

b) a β-oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase.

21. (Amended One Time) The transformed *Candida maltosa* strain of Claim 20 wherein the enhanced alkane hydroxylating activity of a) arises from DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37).

22. A transformed *Candida maltosa* strain SW84/87.2 identified as ATCC 74430.

23. The method of Claims 1, 8, 14, or 19 wherein the at least one C_6 to C_{22} straight chain hydrocarbon is selected from the group consisting of hexane, heptane, octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, reneicosane, docosane and their respective mono-carboxylic acids and esters.

25. A DNA fragment comprising a) a first *Candida maltosa* promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 monooxygenase and b) a second *Candida maltosa* promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 reductase.

26. (Amended One Time) A DNA fragment comprising a) a first *Candida maltosa* PGK promoter which is operably linked to a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)) and b) a second *Candida maltosa* PGK promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 reductase.

27. A plasmid selected from the group consisting of pSW84 and pSW87.